Ciba Specialty Chemicals Corporation
USA

Textile Dyes

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8EHQ-1297-14091

8EHQ-97-14091

Ciba

December 15, 1997

EXPRESS MAIL
REQUESTED

88980000060

Document Processing Center (7407)
(Attn.: Section 8(e) Coordinator)
Office of Pollution Prevention and Toxics
U. S. Environmental Protection Agency
401 M Street, SW
Washington, DC 20460

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RE: **TSCA Section 8(e) Notice; Sensitization and Mutagenicity Results with Disperse Orange 30**

Dear Section 8(e) Coordinator:

There is no confidential business information contained in this letter.

In accordance with EPA's March 16, 1978, policy statement on Section 8(e) reporting under the Toxic Substances Control Act and EPA's June 1991 TSCA Section 8(e) Reporting Guide, Ciba Specialty Chemicals Textile Dyes Division, wishes to bring to your attention sensitization results from a Guinea Pig Maximization Test, and mutagenicity results from a Salmonella typhimurium Reverse Mutation Assay. Chemically, Disperse Orange 30 is Propanenitrile, 3-{{2-(acetyloxy)ethyl}{4-{(2, 6-dichoro-4-nitrophenyl)azo}phenyl}amino}- (CAS No. 5261-31-4).

The allergenic potential of the test substance, Disperse Orange 30, was assessed in albino guinea pigs. Results of this study clearly indicate Disperse Orange 30 induced allergic skin responses in the Guinea pig: 10 of 10 animals showed grade 1 or 2 sensitization responses. The conclusion is clear that the test substance is a sensitizer under the study conditions.

The bacterial mutagenicity assay showed an increased rate of revertants in two of the test strains, but only one strain (TA98) showed revertant colony numbers well in excess of the positive control rate.

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OPPT NCIC

4050 Premier Drive
High Point, NC 27265

Tel. 910 801 2500

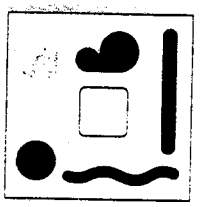
A copy of the final reports entitled "Contact Hypersensitivity to FAT 36141 in Albino Guinea Pigs Maximization-Test"(48 pp), and "Salmonella Typhimurium Reverse Mutation Assay with FAT 36141"(30 pp) is enclosed.

Please contact the undersigned if you require additional information.

Very truly yours,

Brett A. Rajkumar

Brett A. Rajkumar
8e5127.doc/bar
Encl.



CCR PROJECT 592304

Project ID of the Contracting Institute: RCC 664650

SALMONELLA TYPHIMURIUM REVERSE MUTATION ASSAY

WITH

FAT 36´141/C

REPORT

Study Completion Date:
October 27, 1997

RCC
Group

COPY OF GLP CERTIFICATE

HESSISCHES MINISTERIUM FÜR
UMWELT, ENERGIE, JUGEND,
FAMILIE UND GESUNDHEIT

GLP-Bescheinigung

Bescheinigung

Hiermit wird bestätigt, daß die Prüfeinrichtung(en)
CCR Cytotest Cell Research GmbH & Co. KG
in 64380 Roßdorf, In den Leppsteinswiesen19
(Ort, Anschrift)
der RCC/CCR Holding Verwaltungs GmbH
(Firma)
am 05./06./07. April 1995
(Datum)

von der für die Überwachung zuständigen Behörden über die Einhaltung der Grundsätze der Guten Laborpraxis inspiziert worden ist (sind).

Es wird hiermit bestätigt, daß folgende Prüfungen in dieser Prüfeinrichtung nach den Grundsätzen der Guten Laborpraxis durchgeführt werden.

Certificate

It is hereby certified that the test facility(ies)
CCR Cytotest Cell Research GmbH & Co. KG
in 64380 Roßdorf, In den Leppsteinswiesen 19
(location, address)
of RCC/CCR Holding Verwaltungs GmbH
(company name)
on 05./06./07. April 1995
(date)

was (were) inspected by the competent authority regarding compliance with the Principles of Good Laboratory Practice.

It is hereby certified that studies in this test facility are conducted in compliance with the Principles of Good Laboratory Practice.

Prüfkategorie nach § 19 d Abs. 3 Chemikaliengesetz in der Fassung vom 29.Juli 1994 (BGBl. I S. 1703), zuletzt geändert am 27. September 1994 (BGBl. I S. 2705) in Verbindung mit der Allgemeinen Verwaltungsvorschrift zum Verfahren der behördlichen Überwachung der Einhaltung der Grundsätze der Guten Laborpraxis vom 21. Oktober 1990 (BAns. 204 a vom 31.10.1990):

Toxikologische Eigenschaften	Toxicological properties

Prüfkategorie gemäß OECD Panel on Good Laboratory Practice (January 1992)

Prüfungen auf toxikologische Eigenschaften	Toxicity studies
Prüfungen auf mutagene Eigenschaften (in vitro, in vivo)	Mutagenicity studies

Im Auftrag

Dr. Hecker

(Dr. Hecker) Wiesbaden, den 2. August 1995

CONTENTS

PREFACE

General

Sponsor:	Ciba Spezialitätenchemie AG Ecotox TD 2.5 CH-4002 Basel
Study Monitor:	E. Rüdin; RCC Registration & Consulting Co. Ltd. CH-4452 Itingen
Testing Facility:	**C C R** CYTOTEST CELL RESEARCH GMBH & Co. KG In den Leppsteinswiesen 19 D-64380 Roßdorf
CCR Project No.:	592304
Contracting Institute:	R C C REGISTRATION AND CONSULTING COMPANY LTD; CH-4452 Itingen
RCC Project No.:	664650
Test Article:	FAT 36'141/C
CCR Test Article No.:	S1335 11
Title:	Salmonella typhimurium Reverse Mutation Assay with FAT 36'141/C

Project Staff

Study Director:	Dr. Hans-Eric Wollny
Management:	Markus Arenz
Quality Assurance Unit:	Frauke Hermann

Schedule

Date of Protocol:	July 09, 1997
Start of Pre-Experiment:	September 12, 1997
End of Pre-Experiment:	September 15, 1997
Start of Experiment I:	September 12, 1997
End of Experiment I:	September 29, 1997
Start of Experiment II:	October 09, 1997
End of Experiment II:	October 13, 1997
Date of Draft:	October 14, 1997
Date of Final Report:	October 27, 1997

Project Staff Signatures

Study Director

Dr. Hans-Eric Wollny

Wollny

Date: October 27, 1997

Management

Markus Arenz

ppa Wilke

Date: October 27, 1997

Quality Assurance

The study was performed in compliance with:

„ Chemikaliengesetz" (Chemicals Act) of the Federal Republic of Germany, „ Anhang 1" (Annexe 1) dated July 25, 1994 („BGBl. I 1994", pp. 1703)

"The OECD Principles of Good Laboratory Practice", Paris 1981.

Guidelines

This study followed the procedures indicated by the following internationally accepted guidelines and recommendations:

First Addendum to OECD Guidelines for Testing of Chemicals,
Section 4, No. 471, "Salmonella typhimurium, Reverse Mutation Assay", adopted May 26, 1983 and
EEC Directive 92/69, L 383 A, Annexe V, B 14, dated December 29, 1992

Archiving

C C R, D-64380 Roßdorf will archive the following data for 30 years:

Raw data, protocol, and a copy of the report.

The following sample will be archived for at least 2 years following the date on which the report is audited by the Quality Assurance Unit and also at least until the next inspection of CCR Cytotest Cell Research by the GLP-authority:

sample of the test article

If there are no other instructions by the sponsor the raw data and the above mentioned material will be discarded at the end of the archiving period.

STATEMENT OF COMPLIANCE

Project Number:	592304
Test Article:	FAT 36'141/C
Study Director:	Dr. Hans-Eric Wollny
Title:	Salmonella Typhimurium Reverse Mutation Assay with FAT 36'141/C

This study performed in the testing facility of **C C R** was conducted in compliance with Good Laboratory Practice Regulations.

„ Chemikaliengesetz" (Chemicals Act) of the Federal Republic of Germany, „ Anhang 1" (Annexe 1) dated July 25, 1994 („BGBl. I 1994", pp. 1703)

"The OECD Principles of Good Laboratory Practice", Paris 1981.

There were no circumstances that may have affected the quality or integrity of the study.

Study Director

C C R
Dr. Hans-Eric Wollny

Wollny

Date: October 29, 1997

QUALITY ASSURANCE UNIT

C C R, Cytotest Cell Research GmbH & Co. KG,
In den Leppsteinswiesen 19,
D-64380 Roßdorf

Statement

Project Number:	592304
Test Article:	FAT 36'141/C
Study Director:	Dr. Hans-Eric Wollny
Title:	Salmonella Typhimurium Reverse Mutation Assay with FAT 36'141/C

This report was audited by the Quality Assurance Unit and the conduct of this study was inspected on the following dates.

Dates and phases of QAU Inspections/ Audits		Dates of Reports to the Study Director and to Management	
Protocol Audit:	July 11, 1997	July	11, 1997
Process Inspection:	October 08, 1997	October	08, 1997
Draft Audit:	October 17, 1997	October	17, 1997

Head of Quality Assurance Unit — Frauke Hermann

F. Hermann

Date: October 28, 1997

SUMMARY OF RESULTS

This study was performed to investigate the potential of FAT 36'141/C to induce gene mutations according to the plate incorporation test using the Salmonella typhimurium strains TA 1535, TA 1537, TA 98, and TA 100.

The assay was performed in two independent experiments both with and without liver microsomal activation. Each concentration, including the controls, was tested in triplicate. The test article was tested at the following concentrations:

33; 100; 333; 1000; 2500; and 5000 µg/plate (active ingredient)

In experiment I, toxic effects evident as a reduction in the number of revertants, occurred at the highest concentration in strain TA 1535 with S9 mix and in strain TA 1537 without S9 mix. In experiment II, toxic effects occurred at the highest concentration in strains TA 1535 and TA 1537 without S9 mix.

The plates incubated with the test article showed normal background growth up to 5000 µg/plate with and without S9 mix in all strains used.

In both experiments, substantial and dose dependent increases in revertant colony numbers were observed following treatment with FAT 36'141/C with and without metabolic activation.

Appropriate reference mutagens were used as positive controls and showed a distinct increase of induced revertant colonies.

Conclusion

In conclusion, it can be stated that during the described mutagenicity test and under the experimental conditions reported, the test article induced gene mutations by base pair changes and frameshifts in the genome of the strains used.

Therefore, FAT 36'141/C is considered to be mutagenic in this Salmonella typhimurium reverse mutation assay.

OBJECTIVE

Aims of the Study

The experiments were performed to assess the potential of the test article to induce gene mutations by means of two independent Salmonella typhimurium reverse mutation assays. Experiment I was performed as a plate incorporation assay. Since a positive result was obtained in this experiment, experiment II was performed as a plate incorporation assay as well.

Reasons for the Study

The most widely used assays for detecting gene mutations are those using bacteria. They are relatively simple and rapid to perform, and give reliable data on the ability of an agent to interact with DNA and produce mutations.

Reverse mutation assays determine the frequency with which an agent reverses or suppresses the effect of the forward mutation. The genetic target presented to an agent is therefore small, specific and selective. Several bacterial strains, or a single strain with multiple markers are necessary to overcome the effects of mutagen specificity. The reversion of bacteria from growth-dependence on a particular amino acid to growth in the absence of that amino acid (reversion from auxotrophy to prototrophy) is the most widely used marker.

The Salmonella typhimurium histidine (his) reversion system measures $his^- \rightarrow his^+$ reversions. The S. typhimurium strains are constructed to differentiate between base pair (TA 1535, TA 100) and frameshift (TA 1537, TA 98) mutations.

According to the direct plate incorporation method the bacteria are exposed to the test article with and without metabolic activation and plated on selective medium. After a suitable period of incubation, revertant colonies are counted.

To establish a dose response effect six dose levels with adequately spaced concentrations were tested. The maximum dose level was 5000 µg/plate (active ingredient).

To validate the test, reference mutagens are tested in parallel to the test article.

MATERIALS AND METHODS

Test Article

The test article and the information concerning the test article were provided by the sponsor.

Name:	FAT 36'141/C
Batch No.:	not indicated by the sponsor
EN No.:	007581.A7
Aggregate State at Room Temperature:	solid
Colour:	red
Purity:	42.3 % (active ingredient)
Stability in Solvent:	stable at room temperature and at 37° C under test conditions in water, PEG 400, phys. saline, FCA/NaCl-solution, and CMC solution
Storage:	room temperature
Expiration Date:	June, 2002

On the day of the experiment, the test article FAT 36'141/C was dissolved in DMSO. The solvent was chosen because of its solubility properties and its relative nontoxicity to the bacteria.

The test article precipitated weakly at 2500 and 5000 µg/plate in the overlay agar. The undissolved particles of the test article had no influence on the data recording.

Controls

Negative Controls

Concurrent untreated and solvent controls were performed.

Positive Control Substances

Without metabolic activation

Strains:	TA 1535, TA 100
Name:	sodium azide, NaN_3
Supplier:	SERVA, D-69042 Heidelberg
Catalogue No.:	30175
Purity:	at least 99 %
Dissolved in:	water deionised
Concentration:	10 µg/plate

Strains:	TA 1537, TA 98
Name:	4-nitro-o-phenylene-diamine, 4-NOPD
Supplier:	SIGMA, D-82041 Deisenhofen
Catalogue No.:	N 9504
Purity:	> 99.9 %
Dissolved in:	DMSO
Concentration:	10 µg/plate in TA 98, 50 µg/plate in TA 1537

With metabolic activation

Strains:	TA 1535, TA 1537, TA 98, TA 100
Name:	2-aminoanthracene, 2-AA
Supplier:	SIGMA, D-82041 Deisenhofen
Catalogue No.:	A 1381
Purity:	97.5 %
Dissolved in:	DMSO
Concentration:	2.5 µg/plate

The stability of the positive control substances in solution was unknown but a mutagenic response in the expected range is sufficient evidence of biological stability. The dilutions of the stock solutions were prepared on the day of the experiment and used immediately.

Test System

Characterisation of the Salmonella typhimurium Strains

The histidine dependent strains are derived from S. typhimurium strain LT2 through a mutation in the histidine locus. Additionally due to the "deep rough" (rfa-minus) mutation they possess a faulty lipopolysaccharide envelope which enables substances to penetrate the cell wall more easily. A further mutation causes a reduction in the activity of an excision repair system. The latter alteration includes mutational processes in the nitrate reductase and biotin genes produced in a UV-sensitive area of the gene named "uvrB-minus". In the strains TA 98 and TA 100 the R-factor plasmid pKM 101 carries the ampicillin resistance marker.

In summary, the mutations of the TA strains used in this study can be described as follows:

Salmonella typhimurium

TA1537: his C 3076; rfa$^-$; uvrB$^-$:	frame shift mutations
TA 98: his D 3052; rfa$^-$; uvrB$^-$;R-factor:	" " "
TA1535: his G 46; rfa$^-$; uvrB$^-$:	base-pair substitutions
TA 100: his G 46; rfa$^-$; uvrB$^-$;R-factor:	" "

Regular checking of the properties of the strains regarding the membrane permeability, ampicillin- and tetracycline-resistance as well as spontaneous mutation rates is performed in the laboratory of **C C R** according to Ames et al. (1). In this way it was ensured that the experimental conditions set down by Ames were fulfilled.

The bacterial strains TA 1535, TA 98, and TA 100 were obtained from Ames (University of California, 94720 Berkeley, U.S.A.). The bacterial strain TA 1537 was obtained from BASF (D-67063 Ludwigshafen).

Storage

The strain cultures were stored as stock cultures in ampoules with nutrient broth + 5 % DMSO (MERCK, D-64293 Darmstadt) in liquid nitrogen.

Precultures

From the thawed ampoules of the strains 0.5 ml bacterial suspension was transferred into 250 ml Erlenmeyer flasks containing 20 ml nutrient medium. A solution of 20 µl ampicillin (25 µg/ml) was added to the strains TA 98 and TA 100. This nutrient medium contains per litre:

8 g Merck Nutrient Broth (MERCK, D-64293 Darmstadt)
5 g NaCl (MERCK, D-64293 Darmstadt)

The bacterial culture was incubated in a shaking water bath for 8 hours at 37° C.

Selective Agar

The plates with the minimal agar were obtained from E. Merck, D-64293 Darmstadt.

Overlay Agar

The overlay agar contains per litre:

6.0 g MERCK Agar Agar*
6.0 g NaCl*
10.5 mg L-Histidine x HCl x H_2O*
12.2 mg Biotin*

* (MERCK, D-64293 Darmstadt)

Sterilisations were performed at 121° C in an autoclave.

Mammalian Microsomal Fraction S9 Mix

The bacteria used in these assays do not possess the enzyme systems, which, in mammals, are known to convert promutagens into active DNA damaging metabolites. In order to overcome this major drawback an exogenous metabolic system is added in form of mammalian microsome enzyme activation mixture.

S9 (Preparation by C C R)

The S9 liver microsomal fraction was obtained from the livers of 8 - 12 weeks old male rats, strain Wistar HanIbm (BRL, CH-4414 Füllinsdorf; weight approx. 220 - 320 g) which received daily applications of 80 mg/kg b.w. Phenobarbital i.p. dissolved in deionised water (Desitin; D-22335 Hamburg) and β-Naphthoflavone orally dissolved in corn oil (Aldrich, D-89555 Steinheim) on three subsequent days. The livers were prepared 24 hours after the last treatment.

After cervical dislocation the livers of the animals were removed, washed in 150 mM KCl and homogenised. The homogenate was diluted 1+3 in KCl and centrifuged at 9,000 g for 10 minutes at 4° C. A stock of the supernatant containing the microsomes was frozen in ampoules and stored at -80° C. Small numbers of the ampoules are kept at -20° C for up to one week before use. The protein content was determined using an analysis kit of Bio-Rad Laboratories, D-80939 München (Bio-Rad protein assay, Catalogue No. 5000006).

The protein concentration in the S9 preparation was 24.7 mg/ml (lot 030797).

S9 Mix

Before the experiment an appropriate quantity of S9 supernatant was thawed and mixed with S9 co-factor solution. The amount of S9 supernatant was 15% v/v. The composition of the co-factor solution was chosen to yield the following concentrations in the S9 mix:

8 mM $MgCl_2$
33 mM KCl
5 mM Glucose-6-phosphate
5 mM NADP

in 100 mM sodium-ortho-phosphate-buffer, pH 7.4.

During the experiment the S9 mix was stored in an ice bath. The S9 mix preparation was performed according to Ames et al.(2).

Pre-Experiment for Toxicity

To evaluate the toxicity of the test article a pre-experiment was performed with strains TA 98 and TA 100. Eight concentrations were tested for toxicity and mutation induction with each 3 plates. The experimental conditions in this pre-experiment were the same as described for the experiment I below (plate incorporation test).

Toxicity of the test article can be evidenced by a reduction in the number of spontaneous revertants or a clearing of the bacterial background lawn.

Dose Selection

Based upon the results of the pre-experiment the concentrations applied in the main experiments were chosen.

The maximum concentration was 5000 µg/plate (active ingredient). The concentration range included two logarithmic decades. In this study six adequately spaced concentrations were tested. Two independent experiments were performed.

As the results of the pre-experiment were in accordance with the criteria described below (EVALUATION OF RESULTS), these data are reported as a part of the main experiment I.

According to the dose selection criteria the test article was tested at the following concentrations:

33; 100; 333; 1000; 2500; and 5000 µg/plate (active ingredient)

Experimental Performance

For each strain and dose level, including the controls three plates were used.

The following materials were mixed in a test tube and poured onto the selective agar plates:

100 µl	Test solution at each dose level, solvent (negative control) or reference mutagen solution (positive control),
500 µl	S9 mix (for test with metabolic activation) or S9 mix substitution buffer (for test without metabolic activation),
100 µl	Bacteria suspension (cf. test system, pre-culture of the strains),
2000µl	Overlay agar

After solidification the plates were incubated upside down for at least 48 hours at 37° C in the dark.

Data Recording

The colonies were counted using the AUTOCOUNT (Artek Systems Corporation, BIOSYS GmbH, D-61184 Karben). The counter was connected to an IBM AT compatible PC with printer which printed out both, the individual and mean values of the plates for each concentration together with standard deviations and enhancement factors as compared to the spontaneous reversion rates (see tables of results). Due to the intense colour of the test article the colonies were counted manually from 2500 µg/plate up to 5000 µg/plate.

Evaluation of Results

The generally accepted conditions for the evaluation of the results are:

- corresponding background growth on both negative control and test plates
- normal range of spontaneous reversion rates.

Range of spontaneous reversion frequencies * (3)			
1535	1537	98	100
10 - 29	5 - 28	15 - 57	77 - 189

A test article is considered positive if either a biologically relevant and reproducible dose related increase in the number of revertants or a biologically relevant and reproducible increase for at least one test concentration is induced.

A test article producing neither a biologically relevant and reproducible dose related increase in the number of revertants nor a biologically relevant and reproducible positive response at any one of the test points is considered non-mutagenic in this system.

A biologically relevant response is described as follows:

A test article is considered mutagenic if the number of reversions is at least twice the spontaneous reversion rate in strains TA 98 and TA 100 or thrice on TA 1535 and TA 1537 (3, 4).

Also, a dose-dependent and reproducible increase in the number of revertants is regarded as an indication of possibly existing mutagenic potential of the test article regardless whether the highest dose induced the criteria described above or not.

* These results are referring to the negative control group without metabolic activation and represent our historical control range since 1993

DISCUSSION OF RESULTS

The test article FAT 36'141/C was assessed for its potential to induce gene mutations according to the plate incorporation test using the Salmonella typhimurium strains TA 1535, TA 1537, TA 98, and TA 100.

The assay was performed in two independent experiments both with and without liver microsomal activation. Each concentration and the controls, were tested in triplicate. The test article was tested at the following concentrations:

33, 100; 333; 1000; 2500; and 5000 µg/plate (active ingredient)

In experiment I, toxic effects evident as a reduction in the number of revertants occurred at the highest concentration in strain TA 1535 with S9 mix and in strain TA 1537 without S9 mix. In experiment II, toxic effects occurred at the highest concentration in strains TA 1535 and TA 1537 without S9 mix.

The plates incubated with the test article showed normal background growth up to 5000 µg/plate with and without S9 mix in all strains used.

In both experiments, substantial and dose dependent increases in revertant colony numbers were observed following treatment with FAT 36'141/C with and without metabolic activation in strains TA 1537, TA 98 and TA 100. The number of colonies reached or exceeded the threshold of twice (strains TA 98 and TA 100) and thrice (strain TA 1537) the number of the corresponding solvent control at concentrations as low as 33 µg/plate and above. In experiment I a dose dependent increase in revertant colony numbers was observed in strain TA 1535 with and without S9 mix. In the absence of metabolic activation the threshold of thrice the number of the corresponding solvent control was not quite reached. In the presence of metabolic activation the threshold was exceeded at 2500 µg/plate. In experiment II, a dose dependent increase was observed in strains TA 1535 and TA 1537 in the presence of metabolic activation but the threshold was not reached. In the absence of metabolic activation the threshold was exceeded at 2500 µg/plate in strain TA 1537.

Appropriate reference mutagens were used as positive controls. They showed a distinct increase in induced revertant colonies.

In conclusion, it can be stated that during the described mutagenicity test and under the experimental conditions reported, the test article induced gene mutations by base pair changes and frameshifts in the genome of the strains used.

REFERENCES

1. Ames, B.N., Maron D.M. (1983)
Revised methods for the Salmonella mutagenicity test
Mutation Res. 113, 173-215

2. Ames, B.N., J. McCann, and E. Yamasaki (1977)
Methods for detecting carcinogens and mutagens with the Salmonella/mammalian microsome mutagenicity test
In: B.J. Kilbey et al. (Eds.)"Handbook of Mutagenicity Test Procedures" Elsevier, Amsterdam, 1-17

3. de Serres F.J. and M.D. Shelby (1979)
Recommendations on data production and analysis using the Salmonella/microsome mutagenicity assay
Mutation Res. 64, 159-165

4. Hollstein,M., J. McCann, F.A. Angelosanto and W.W. Nichols (1979)
Short-term tests for carcinogens and mutagens
Mutation Res. 65, 133-226

ANNEX: TABLES OF RESULTS

Pre-Experiment for Toxicity

To evaluate the toxicity of the test article a pre-study was performed with strains TA 98 and TA 100. The results are given in the following table:

Table 1:

Substance	Concentration per plate µg	Revertants per plate TA 98		TA 100	
		–	+	–	+
Negative control	–	27	32	133	137
Solvent control	–	26	32	122	110
4-NOPD	10.0	717	/	/	/
Sodium azide	10.0	/	/	1001	/
2-aminoanthracene	2.5	/	868	/	485
test article	3	38	37	107	119
	10	89	69	104	122
	33	178	144	106	124
	100	362	567	133	170
	333	581	1186	156	254
	1000	747	1536	171	306
	2500	922	1631	200	328
	5000	842	1559	247	400

– = without S9 mix
+ = with S9 mix
/ = not performed

The plates with the test article showed normal background growth up to 5000 µg/plate in strain TA 98 and TA 100.

According to the dose selection criteria, the test article was tested at the following concentrations:

33; 100; 333; 1000; 2500; and 5000 µg/plate

Experiment I: Plate Incorporation Test

Test article: FAT 36'141/C

S9 mix from : Rat liver (Batch R 030797)

Test strain: TA 1535

without S9 mix

Concentration µg/plate	Plate 1	2	3	Revertants / plate mean	s.d.	factor*
Negative Control	9	5	11	8	3.1	
Solvent Control	9	7	18	11	5.9	1.0
Positive Control#	915	949	1033	966	60.7	85.2
33	13	20	17	17	3.5	1.5
100	16	19	19	18	1.7	1.6
333	17	17	12	15	2.9	1.4
1000	15	18	15	16	1.7	1.4
2500	19	28	22	23	4.6	2.0
5000	29	33	31	31	2.0	2.7

with S9 mix

Concentration µg/plate	Plate 1	2	3	Revertants / plate mean	s.d.	factor*
Negative Control	14	14	12	13	1.2	
Solvent Control	14	11	13	13	1.5	1.0
Positive Control##	247	267	242	252	13.2	19.9
33	18	12	9	13	4.6	1.0
100	15	21	22	19	3.8	1.5
333	18	18	19	18	0.6	1.4
1000	31	34	28	31	3.0	2.4
2500	26	45	46	39	11.3	3.1
5000	21	17	22	20	2.6	1.6

$$\text{* enhancement factor} = \frac{\Sigma \text{ revertants / concentr. test article}}{\Sigma \text{ revertants / solvent control}}$$

\# sodium azide 10 µg/plate

\## 2-aminoanthracene 2.5 µg/plate

Experiment I: Plate Incorporation Test

Test article: FAT 36'141/C

S9 mix from : Rat liver (Batch R 030797)

Test strain: TA 1537

without S9 mix

Concentration µg/plate	Plate 1	2	3	Revertants / plate mean	s.d.	factor*
Negative Control	10	12	7	10	2.5	
Solvent Control	12	11	12	12	0.6	1.0
Positive Control#	79	105	109	98	16.3	8.4
33	19	11	12	14	4.4	1.2
100	11	18	21	17	5.1	1.4
333	25	24	19	23	3.2	1.9
1000	47	46	52	48	3.2	4.1
2500	77	84	68	76	8.0	6.5
5000	22	43	40	35	11.4	3.0

with S9 mix

Concentration µg/plate	Plate 1	2	3	Revertants / plate mean	s.d.	factor*
Negative Control	13	13	15	14	1.2	
Solvent Control	12	13	8	11	2.6	1.0
Positive Control##	167	173	170	170	3.0	15.5
33	14	14	34	21	11.5	1.9
100	33	43	35	37	5.3	3.4
333	54	47	57	53	5.1	4.8
1000	70	77	71	73	3.8	6.6
2500	80	79	86	82	3.8	7.4
5000	131	128	144	134	8.5	12.2

$$\text{* enhancement factor} = \frac{\Sigma \text{ revertants / concentr. test article}}{\Sigma \text{ revertants / solvent control}}$$

\# 4-nitro-o-phenylene-diamine 50 µg/plate

\## 2-aminoanthracene 2.5 µg/plate

Experiment I: Plate Incorporation Test

Test article: FAT 36'141/C

S9 mix from : Rat liver (Batch R 030797)

Test strain: TA 98

without S9 mix

Concentration µg/plate	Plate 1	2	3	Revertants / plate mean	s.d.	factor*
Negative Control	26	26	30	27	2.3	
Solvent Control	21	32	25	26	5.6	1.0
Positive Control#	766	703	683	717	43.3	27.6
33	196	184	153	178	22.2	6.8
100	350	361	374	362	12.0	13.9
333	567	586	590	581	12.3	22.3
1000	710	641	890	747	128.6	28.7
2500	809	1011	947	922	103.2	35.5
5000	731	978	816	842	125.5	32.4

with S9 mix

Concentration µg/plate	Plate 1	2	3	Revertants / plate mean	s.d.	factor*
Negative Control	30	29	38	32	4.9	
Solvent Control	33	31	31	32	1.2	1.0
Positive Control##	1119	706	778	868	220.6	27.4
33	95	167	170	144	42.5	4.5
100	616	527	558	567	45.2	17.9
333	1109	1354	1094	1186	146.0	37.4
1000	1421	1605	1582	1536	100.3	48.5
2500	1594	1632	1666	1631	36.0	51.5
5000	1474	1489	1714	1559	134.4	49.2

$$\text{* enhancement factor} = \frac{\Sigma \text{ revertants / concentr. test article}}{\Sigma \text{ revertants / solvent control}}$$

\# 4-nitro-o-phenylene-diamine 10 µg/plate

\## 2-aminoanthracene 2.5 µg/plate

Experiment I: Plate Incorporation Test

Test article: FAT 36'141/C

S9 mix from : Rat liver (Batch R 030797)

Test strain: TA 100

without S9 mix

Concentration µg/plate	Plate 1	2	3	Revertants / plate mean	s.d.	factor*
Negative Control	135	122	143	133	10.6	
Solvent Control	108	123	136	122	14.0	1.0
Positive Control#	1010	942	1051	1001	55.1	8.2
33	125	91	103	106	17.2	0.9
100	135	140	125	133	7.6	1.1
333	170	164	135	156	18.7	1.3
1000	161	190	163	171	16.2	1.4
2500	207	196	196	200	6.4	1.6
5000	249	255	237	247	9.2	2.0

with S9 mix

Concentration µg/plate	Plate 1	2	3	Revertants / plate mean	s.d.	factor*
Negative Control	136	139	137	137	1.5	
Solvent Control	116	106	109	110	5.1	1.0
Positive Control##	441	501	512	485	38.2	4.4
33	142	128	102	124	20.3	1.1
100	180	161	169	170	9.5	1.5
333	262	234	265	254	17.1	2.3
1000	243	354	322	306	57.1	2.8
2500	314	329	340	328	13.1	3.0
5000	389	412	399	400	11.5	3.6

$$\text{* enhancement factor} = \frac{\Sigma \text{ revertants / concentr. test article}}{\Sigma \text{ revertants / solvent control}}$$

sodium azide 10 µg/plate

2-aminoanthracene 2.5 µg/plate

Experiment II: Plate Incorporation Test

Test article: FAT 36'141/C

S9 mix from : Rat liver (Batch R 030797)

Test strain: TA 1535

without S9 mix

Concentration µg/plate	Plate 1	2	3	Revertants / plate mean	s.d.	factor*
Negative Control	11	11	13	12	1.2	
Solvent Control	14	14	16	15	1.2	1.0
Positive Control#	755	434	527	572	165.2	39.0
33	13	12	11	12	1.0	0.8
100	12	5	10	9	3.6	0.6
333	16	16	15	16	0.6	1.1
1000	14	14	18	15	2.3	1.0
2500	13	8	9	10	2.6	0.7
5000	8	7	3	6	2.6	0.4

with S9 mix

Concentration µg/plate	Plate 1	2	3	Revertants / plate mean	s.d.	factor*
Negative Control	14	15	18	16	2.1	
Solvent Control	10	15	11	12	2.6	1.0
Positive Control##	213	227	230	223	9.1	18.6
33	21	17	15	18	3.1	1.5
100	10	22	13	15	6.2	1.3
333	21	11	13	15	5.3	1.3
1000	16	17	10	14	3.8	1.2
2500	21	31	11	21	10.0	1.8
5000	30	18	34	27	8.3	2.3

$$\text{* enhancement factor} = \frac{\Sigma \text{ revertants / concentr. test article}}{\Sigma \text{ revertants / solvent control}}$$

\# sodium azide 10 µg/plate

\## 2-aminoanthracene 2.5 µg/plate

Experiment II: Plate Incorporation Test

Test article: FAT 36'141/C

S9 mix from : Rat liver (Batch R 030797)

Test strain: TA 1537

without S9 mix

Concentration µg/plate	Plate 1	2	3	Revertants / plate mean	s.d.	factor*
Negative Control	13	12	12	12	0.6	
Solvent Control	6	9	5	7	2.1	1.0
Positive Control#	90	126	137	118	24.6	17.7
33	22	9	15	15	6.5	2.3
100	5	11	7	8	3.1	1.2
333	12	15	17	15	2.5	2.2
1000	20	15	19	18	2.6	2.7
2500	44	25	32	34	9.6	5.1
5000	3	9	1	4	4.2	0.7

with S9 mix

Concentration µg/plate	Plate 1	2	3	Revertants / plate mean	s.d.	factor*
Negative Control	19	14	11	15	4.0	
Solvent Control	20	17	14	17	3.0	1.0
Positive Control##	98	92	101	97	4.6	5.7
33	15	22	10	16	6.0	0.9
100	17	16	8	14	4.9	0.8
333	29	25	41	32	8.3	1.9
1000	29	43	18	30	12.5	1.8
2500	37	60	43	47	11.9	2.7
5000	35	64	45	48	14.7	2.8

$$\text{* enhancement factor} = \frac{\Sigma \text{ revertants / concentr. test article}}{\Sigma \text{ revertants / solvent control}}$$

\# 4-nitro-o-phenylene-diamine 50 µg/plate

\## 2-aminoanthracene 2.5 µg/plate

Experiment II: Plate Incorporation Test

Test article: FAT 36'141/C

S9 mix from : Rat liver (Batch R 030797)

Test strain: TA 98

without S9 mix

Concentration µg/plate	Plate 1	2	3	Revertants / plate mean	s.d.	factor*
Negative Control	22	26	16	21	5.0	
Solvent Control	16	21	15	17	3.2	1.0
Positive Control#	634	650	605	630	22.8	36.3
33	51	49	43	48	4.2	2.8
100	141	115	120	125	13.8	7.2
333	201	198	180	193	11.4	11.1
1000	324	318	331	324	6.5	18.7
2500	607	589	593	596	9.5	34.4
5000	721	698	705	708	11.8	40.8

with S9 mix

Concentration µg/plate	Plate 1	2	3	Revertants / plate mean	s.d.	factor*
Negative Control	21	25	19	22	3.1	
Solvent Control	25	28	22	25	3.0	1.0
Positive Control##	1001	1095	958	1018	70.1	40.7
33	58	62	54	58	4.0	2.3
100	164	151	132	149	16.1	6.0
333	198	205	286	230	48.9	9.2
1000	356	398	396	383	23.7	15.3
2500	598	623	605	609	12.9	24.3
5000	782	798	778	786	10.6	31.4

$$\text{* enhancement factor} = \frac{\Sigma \text{ revertants / concentr. test article}}{\Sigma \text{ revertants / solvent control}}$$

\# 4-nitro-o-phenylene-diamine 10 µg/plate

\## 2-aminoanthracene 2.5 µg/plate

Experiment II: Plate Incorporation Test

Test article: FAT 36'141/C

S9 mix from : Rat liver (Batch R 030797)

Test strain: TA 100

without S9 mix

Concentration µg/plate	Plate 1	2	3	Revertants / plate mean	s.d.	factor*
Negative Control	104	96	109	103	6.6	
Solvent Control	92	96	86	91	5.0	1.0
Positive Control#	691	774	565	677	105.2	7.4
33	120	113	99	111	10.7	1.2
100	114	85	131	110	23.3	1.2
333	132	130	145	136	8.1	1.5
1000	152	152	164	156	6.9	1.7
2500	173	185	229	196	29.5	2.1
5000	453	584	140	392	228.1	4.3

with S9 mix

Concentration µg/plate	Plate 1	2	3	Revertants / plate mean	s.d.	factor*
Negative Control	95	147	86	109	32.9	
Solvent Control	152	141	156	150	7.8	1.0
Positive Control##	727	881	835	814	79.1	5.4
33	109	132	124	122	11.7	0.8
100	148	191	117	152	37.2	1.0
333	244	206	254	235	25.3	1.6
1000	297	362	212	290	75.2	1.9
2500	283	342	300	308	30.4	2.1
5000	335	330	339	335	4.5	2.2

$$\text{* enhancement factor} = \frac{\Sigma \text{ revertants / concentr. test article}}{\Sigma \text{ revertants / solvent control}}$$

\# sodium azide 10 µg/plate

\## 2-aminoanthracene 2.5 µg/plate

Summary of Results

Test article: FAT 36'141/C

S9 mix from: Rat liver (Batch R 030797)

without S9 mix

Concentration µg/plate	Revertants/plate mean from three plates							
	TA 1535		TA 1537		TA 98		TA 100	
	I	II	I	II	I	II	I	II
Negative control	8	12	10	12	27	21	133	103
Solvent control	11	15	12	7	26	17	122	91
Positive control#	966	572	98	118	717	630	1001	677
33	17	12	14	15	178	48	106	111
100	18	9	17	8	362	125	133	110
333	15	16	23	15	581	193	156	136
1000	16	15	48	18	747	324	171	156
2500	23	10	76	34	922	596	200	196
5000	31	6	35	4	842	708	247	392

with S9 Mix

Concentration µg/plate	Revertants/plate mean from three plates							
	TA 1535		TA 1537		TA 98		TA 100	
	I	II	I	II	I	II	I	II
Negative control	13	16	14	15	32	22	137	109
Solvent control	13	12	11	17	32	25	110	150
Positive control##	252	223	170	97	868	1018	485	814
33	13	18	21	16	144	58	124	122
100	19	15	37	14	567	149	170	152
333	18	15	53	32	1186	230	254	235
1000	31	14	73	30	1536	383	306	290
2500	39	21	82	47	1631	609	328	308
5000	20	27	134	48	1559	786	400	335

\# Sodium azide (10.0 µg/plate) strains TA 1535 and TA 100

\## 4-nitro-o-phenylene-diamine strains TA 1537 (50 µg/plate) and TA 98 (10.0 µg/plate)
2-aminoanthracene (2.5 µg/plate) strains TA 1535, TA 1537, TA 98, and TA 100

Biometry

A statistical analysis of the data is not required.

Deviations to Protocol

There were no deviations to protocol

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